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SAFETY TESTING OF DENGUE-1 AND DENGUE-3 SEEDS FOR HUMAN CHALLENGES, UNATTENUATED; HEPATITIS A VIRUS, STRAIN HM175

PHASE REPORT

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Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lot 1A of 16 Oct 87 and Lot 1B of 4 Dec 87 was satisfactorily safety-tested in accordance with the guidelines established by the FDA for live and inactivated vaccines as stipulated in 21 CFR, Parts 610.11, 610.12, 630.10-630.17, etc. All testing procedures were carried out following Good Laboratory Practices (GLP) regulations (21 CFR, Part 58). Results are reported herein.

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FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS, PHS, NIH Publications No. 85-23, Revised 1985).

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I. INTRODUCTION

The accompanying protocol is a description of the safety testing of a lot of hepatitis A virus designated as:

Hepatitis A Virus, Strain HM175,
Vaccine FI-2, Lots 1A and 1B

Utilizing the testing procedures herein described, this fluid is considered to have passed satisfactorily all tests for safety including purity. The detailed records with respect to passage history, pool production, virus characterization, pooling and subsequent safety testing may be found in the laboratory notebooks located at:

The Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, DC 20307-5100 - (Dr. Ken Eckels)

The Experimental Virus Vaccine Production Laboratory - Suite #500 - Flow Laboratories, Inc., McLean, VA - (Dr. Louis Potash)

All procedures performed at Flow Laboratories followed Good Laboratory Practices (GLP) regulations (21 CFR, Part 58) and were carried out in accordance with the guidelines established by the FDA for live and inactivated vaccines as found in 21 CFR, Parts 610.11, 610.12, 610.30, 630.10 - 630.18, etc. These procedures are detailed in the following SOPs and recorded on the indicated VVPL Forms:

SOP No.:	400.004 -	Issued	25 Feb 1980,	Revised	18 Feb	1986
	400.005 -	"	25 Feb 1980,	"	18 Feb	1986
	400.006 -	"	25 Feb 1980,	"	18 Feb	1986
	400.007 -	"	25 Feb 1980,	"	18 Feb	1986
	400.008 -	"	12 Apr 1984,	"	18 Feb	1986
	400.009 -	"	3 May 1984,	"	18 Feb	1986
	500.001 -	"	29 Oct 1980,	"	18 Feb	1986
	500.002 -	"	29 Oct 1980,	"	18 Feb	1986
	500.008 -					
VVPL FORM	#003 -	Issued	3 Ap			
	004 -	"	16 Jan 1981,	Revised	21 Mar	1984
	008 -	"	29 Oct 1980,	"	3 May	1984
	016 -	"	15 Jan 1981,	"	13 July	1984
	017 -	"	16 Jan 1981,	"	13 Jan	1986
	019 -	"	8 Oct 1984			
	023 -	"	19 Feb 1986			

II. SYNOPSIS

A. Virus Strain: Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lot 1A of 16 Oct 87 and Lot 1B of 4 Dec 87

B. Treatment/Handling: Prior to safety testing, Lots 1A and 1B pooled & redistributed.

C. Safety Tests on Crude Harvest Fluids:

1.	Sterility: Fluid Thioglycollate (FTM), Tryptone Soya Broth (TSB), Lowenstein-Jensen Egg Medium, Mycoplasma		
	a. Virus Pool	(52 ml)	No Growth
	b. Control Pool (TCF)	(52 ml)	No Growth
2.	Tissue Culture Identity and Purity (Safety): AGMK, PHA, MRC-5, PRK, and Flow 5000.		
	a. Virus Pool	(100 ml)	Satisfactory
	b. Control Pool (TCF)	(100 ml)	Satisfactory
3.	Animal Safety:		
	a. Rabbits: Intradermal and Subcutaneous		
	(1) Virus Pool	(20 ml)	Satisfactory
	(2) Control Pool (TCF)	(20 ml)	Satisfactory
	b. Adult Mice: Intracerebral and I.P.		
	(1) Virus Pool	(11 ml)	Satisfactory
	(2) Control Pool (TCF)	(11 ml)	Satisfactory
	c. Suckling Mice: Intracerebral and I.P.		
	(1) Virus Pool	(2.5 ml)	Satisfactory
	(2) Control Pool (TCF)	(2.5 ml)	Satisfactory
	d. Guinea Pigs: Intracerebral and I.P.		
	(1) Virus Pool	(15.5 ml)	Satisfactory
	(2) Control Pool (TCF)	(15.5 ml)	Satisfactory
4.	Reverse Transcriptase:	(2 ml)	No RT Enzyme

III. DETAILED SUMMARY RELATING TO THE SAFETY TESTING OF CRUDE HEPATITIS A VIRUS, STRAIN HM175, VACCINE FI-2, LOT 1A and LOT 1B: PROPAGATED IN MRC-5 CELL CULTURES

A. Inocula

On February 25, 1988, the following frozen materials were obtained for testing from Dr. K. Eckels, Contracting Officer's Representative, at the Walter Reed Army Institute of Research (WRAIR), Bldg. 501, Washington, DC 20307-5100:

1. HAV HM-175, Vaccine FI-2, Lot 1A, day 31 harvest, unclarified of 16 Oct 1987: 5 x 25 ml vials

2. HAV HM-175, Vaccine FI-2, Lot 1B, day 31 harvest, unclarified of 4 Dec 1987: 5 x 25 ml vials

3. Control Fluid for HAV Vaccine FI-2, Lot 1A, day 31 harvest, unclarified of 16 Oct 1987: 5 x 25 ml vials

4. Control Fluid for HAV Vaccine FI-2, Lot 1B, day 31 harvest, unclarified of 4 Dec 1987: 5 x 25 ml vials

On arrival in this laboratory, all fluids were stored at -70°C, or below. Since it was determined that the subsequent purified vaccine would be a pool of both Lots and since each of the 4 lots consisted of only 125 ml (a volume insufficient to perform all the prescribed tests), it was agreed that the fluids would tested as pools. Therefore, prior to commencing the safety testing, all fluids were thawed with Items #1 & #2 pooled and Items #3 & #4 pooled and then each pool redistributed into the following volumes: 8 x 25 ml; 5 x 5 ml and 5 x 2 ml.

B. Safety Testing Procedures and Results on the Crude, Unclarified Harvest Fluids (SOP No.: 500.008)

1. Microbial Sterility - (VVPL FORM #011)

Aliquots of the bulk frozen fluids were thawed and tested for microbial sterility as follows:

a. Fluid Thioglycollate Medium - FTM - (LOT #35045215): Each of 10 culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus pool and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid pool. An additional 10 cultures were included as uninoculated controls. All cultures were vortex mixed and incubated at 31°C (+ 1°C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 30 culture tubes.

b. Tryptone Soya Broth - TSB - (LOT #35060235): Each of 10 culture culture tubes (9-10 ml medium per tube) was inoculated with 1 ml volumes of the crude virus pool and each of 10 culture tubes was inoculated with 1 ml volumes of the crude control fluid pool. An additional 10 cultures were included as uninoculated controls. All cultures were vortex mixed and incubated at 22°C (+ 2°C) for 21 days with periodic examination for evidence of growth. No growth was observed in any of the 30 culture tubes.

c. Lowenstein-Jensen Egg Medium (BBL - Lot #J8CZKK): Each of 10 culture tubes was inoculated with 0.5 ml of the crude virus pool and each of 10 culture tubes was inoculated with 0.5 ml of the crude control fluid pool. Ten additional culture tubes were included as uninoculated controls. All cultures were incubated at 37°C -- horizontally for the first 24 hours and then vertically for the remainder of the 8-week observation period. Cultures were examined periodically for growth over this 8-week period. No growth was observed in any of the 30 cultures.

The results of the above described Microbial Sterility Assays are summarized in Table I.

d. Mycoplasma Sterility: These assays were performed by the Flow Laboratories' Mycoplasma Testing Laboratory and included both the routine PPLO agar and broth assays and the specific test for the detection of M. hyorhinis. Samples (1 x 25 ml and 1 x 2 ml of both the crude virus pool and of the crude control fluid pool) were submitted for testing. All samples were reported to be negative for mycoplasmas. A copy of this report is appended to this Protocol - (Appendixes - 1 & 2).

2. Identity in Tissue Culture (Serum-Neutralization) - (VVPL FORM #015)

No attempt was made to identity the crude virus pool in tissue cultures.

3. Purity (Safety) in Tissue Cultures - (VVPL FORM #016)

a. Tissue Cultures: "Fully" sheeted flask or roller tube cell cultures were prepared by laboratory personnel. Cultures were maintained on Medium MEM containing 5 to 10% fetal bovine serum (heat-inactivated) plus antibiotics: gentamicin @ 100 mcg/ml; neomycin @ 50 mcg/ml; and amphotericin B (I.V.) @ 2.5 mcg/ml. Cultures were inoculated, refed and subpassaged as indicated below. The following tissue culture systems were utilized:

(1) Tertiary African Green Monkey Kidney (AGMK) ..... MEM + 5% serum
(2) Primary Human Amnion (PHA) ..... MEM + 10% serum
(3) Human Diploid Fibroblast (MRC-5) ..... MEM + 5% serum
(4) Primary Rabbit Kidney (PRK) ..... MEM + 5% serum
(5) Whole Human Embryo Fibroblast (Flow 5000) ..... MEM + 5% serum

b. General Testing Procedures

(1) Crude Virus Fluid

(a) Primary Flask Cultures: Since hepatitis A virus does not produce any discernible CPE in tissue cultures, no attempt was made to neutralize the virus for these studies. A total of 20 ml of the crude virus fluid was tested per tissue culture system wherein each of 2 x 75 cm^2 flask per tissue culture system was inoculated with 10 ml of the pool. Flasks contained approximately 25 ml of maintenance medium at the time of inoculation. Cultures were incubated at 35°C (37°C for PHA) for 14 days with periodic microscopic examination for any signs of CPE and/or cellular degradation. When necessary to maintain the integrity of the cell films, cultures were refed with 35 ml of fresh medium.

(b) Secondary Tube Subcultures: On the 14th day of incubation, the primary cultures were re-examined microscopically and the fluids harvested individually. To each individual harvest was added: 0.1 ml gentamicin (50 mg/ml); 1 ml penicillin-streptomycin solution (5000 units/ml and 5000 mcg/ml, respectively); and 10% of 10X SPG* (v/v). Following mixing, the fluids were incubated at room temperature for 60 minutes and then subpassed into homologous roller tube cultures - 0.5 ml of each harvest into each of 20 tubes. The remainder of the harvest fluids was saved and stored at -75°C, or below. All primary cultures were tested for hemadsorption by the addition of 0.1% guinea pig RBC (in PBS) and incubation at 4°C for a minimum of 30 minutes. All cultures were negative for hemadsorption and for hemagglutination.

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* 10X SPG. sucrose, 2.18 M; KH_2PO_4, 0.038 M; K_2HPO_4, 0.072 M; potassium glutamate, 0.049 M.

Tube cultures (refed with 2 ml of maintenance medium prior to inoculation) were incubated at 35°C (37°C for PHA) for 14 additional days. When necessary to maintain the integrity of the cell films, cultures were refed with 2 ml of fresh medium. Cultures were examined microscopically at periodic intervals and at the end of the incubation period for any signs of CPE. After final examination, tubes were divided - depending on the specific cell system - for additional testing:

AGMK, PHA, MRC-5 and Flow 5000 Tube Cultures: These were divided into 3 groups as follows:

1/4th tested for the presence of hemadsorbing agents,
1/4th fixed and stained with a solution of 5% glutaraldehyde + 1:10 giemsa stain and examined microscopically for any CPE,
1/2 Challenged with Coxsackie A-9 virus (0.2 ml per tube at dilutions noted in the tables) for the detection of non-CPE producing agents and/or latent agents via the interference phenomenon.

PRK Tube Cultures: These were equally divided into 2 groups:

1/2 tested for the presence of hemadsorbing agents,
1/2 fixed and stained with the glutaraldehyde-giemsa stain solution and examined microscopically for any CPE.

No challenge studies were carried out with the Coxsackie A-9 virus since this virus does not produce any discernible CPE in this tissue culture system.

(2) Crude Control Fluid

A total of 20 ml of the crude control fluid was tested per tissue culture system wherein each of 2 x 75 cm^2 flasks per tissue culture system was inoculated with 10 ml of the pool. Cultures were handled in a manner similar to that described above for the crude virus fluid pool.

(3) Uninoculated Cell Lot Controls

Two x 75 cm^2 flasks per tissue culture system were included as uninoculated cell lot controls and were handled in a manner similar to that described above. In addition, an appropriate number of uninoculated roller tube cultures were included as cell lot controls for the secondary tube subcultures.

In all challenge studies, 1 to 4 culture tubes per set were left unchallenged to serve as controls to the challenge virus.

The results of these in vitro Tissue Culture Purity (Safety) tests are summarized in Tables II-A through -E.

4. Animal Safety Tests - (VVPL FORM #004)

a. Adult Rabbits - Test for B-virus and other adventitious agents (SOP No.: 400.004)

Each of 2 New Zealand white rabbits (1500-2500 gms eacn) was inoculated intradermally in multiple sites with a total of 1 ml and subcutaneously with 9 ml of the crude virus pool. In addition, the right cornea was scratched and 0.03 ml of the crude virus fluid was applied. Each of two rabbits was similarly inoculated with the crude control fluid pool with the left cornea scratched and the crude control fluid applied. One additional rabbit was included as an uninoculated control. All the rabbits were observed daily for a total of 28 days for deaths and/or signs of lesions at sites of inoculation and for any signs of illness or distress. All rabbits remained healthy and none exhibited any signs of illness or distress or lesions at the sites of inoculation. This test in adult rabbits was considered satisfactory.

b. Adult Mice - Test for adventitious agents - (SOP No. 400.005)

Each of 20 adult CD-1 mice (15-20 grams each) was inoculated intracerebrally with 0.03 ml and intraperitoneally with 0.5 ml of the crude virus pool and each of 20 adult CD-1 mice was similarly inoculated with the crude control fluid pool. An additional 10 mice were included as uninoculated controls. The mice were observed daily for deaths and/or signs of illness or distress over a 4 week period. All mice (inoculated as well as controls) remained healthy and survived the entire 28-day observation period with no evidence of lymphocytic choriomeningitis virus infection or of any other virus infection. This test in adult mice was considered satisfactory.

c. Suckling Mice - Test for adventitious agents - (SOP No.: 400.005)

Two groups of 20 newborn CD-1 mice from mixed litters (10 per mother - less than 24 hours old) were inoculated intracerebrally with 0.01 ml and intraperitoneally with 0.1 ml as follows: one group with the crude virus pool and one group with the crude control fluid pool. An additional litter of 10 sucklings was included to serve as uninoculated controls. All sucklings were observed daily for 14 days for deaths and/or signs of illness or distress. There were no deaths and no signs of illness or distress recorded for any of the litters over this initial 14-day observation period.

On the 14th day, single pools were prepared of the emulsified tissue (minus skin and viscera) of the following groups: a) virus inoculated sucklings [20]; b) control fluid inoculated sucklings [20]; and c) uninoculated controls [10]. A blind passage into newborn CD-1 mice was made of each of the 3 pools via the intracerebral and intraperitoneal routes: the individual pools from the inoculated sucklings (a and b) into each of 20 newborns and the pool from the uninoculated control sucklings (c) into 10 newborns. An additional litter of 10 sucklings was included as uninoculated controls (d) for this blind passage. All sucklings were observed daily for 14 days for deaths and/or signs of illness or distress. There were no deaths and none of the sucklings exhibited any signs of illness or distress over this final 14-day observation period.

Since none of the inoculated sucklings exhibited any evidence of a transmissible agent or of Coxsackie virus infection or of any viral infection, and since 100% of these inoculated sucklings remained healthy and survived the entire observation period, this test in suckling mice was considered satisfactory.

d. Adult Guinea Pigs - (SOP No.: 400.006)

Test for M. tuberculosis: Each of 3 adult guinea pigs (Hartley Strain, virus free, 350-450 grams each) was inoculated intracerebrally with 0.1 ml and intraperitoneally with 5 ml of the un-neutralized crude virus fluid, and each of 3 guinea pigs was similarly inoculated with the crude control fluid. An additional 3 guinea pigs were included as uninoculated controls. All pigs were observed daily for a period of 6 weeks for deaths and/or any signs of illness or distress. Commencing on day 21, daily rectal temperatures (LED digital thermistor thermometer) were taken and recorded (± 0800 hrs) for all guinea pigs until time of sacrifice. The average temperatures (^{o}C) for the 3 groups of guinea pigs were: 38.27, 38.41, and 38.49 for the virus fluid inoculated; 38.25, 38.29 and 38.43 for the control fluid inoculated; and 38.32, 38.45 and 38.56 for the uninoculated controls. There were no significant rises indicative of either bacterial or viral infection. All guinea pigs appeared healthy and survived the entire 42-day observation period at which time they were necropsied following euthanasia with Halathane. Inspection of the abdominal and thoracic cavities indicated no gross pathological changes. This test in guinea pigs was considered satisfactory.

The results of these in vivo Animal Safety Tests are summarized in Table III - A and - B.

5. Reverse Transcriptase - Assay for the detection of RNA-dependent DNA-polymerase activity

The assay for Reverse Transcriptase was performed by Dr. Allan Tereba at the St. Jude Children's Research Hospital, Memphis, TN. Two ml samples of the clarifed (centrifuged) crude virus pool and crude control fluid pool were submitted for assay. Both samples were reported to be negative for the RT Enzyme and a copy of this report is appended to this Protocol - (Appendix - 3).

Table I. Microbial Sterility Test Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool.

Culture Medium	No.	Vol. per culture (ml)	Temperature	Date On Test	Date Off Test	Results
Fluid Thioglycollate						
(FTM) LOT #35045215	10	-----	31°C (±1°C)	3/10/88	3/31/88	No Growth
Virus Pool	10	1.0		|	|	No Growth
Control Pool	10	1.0		3/10/88	3/31/88	No Growth
Tryptone Soya Broth						
(TSB) LOT #35060235	10	-----	22°C (±2°C)	3/10/88	3/31/88	No Growth
Virus Pool	10	1.0		|	|	No Growth
Control Pool	10	1.0		3/10/88	3/31/88	No Growth
Lowenstein-Jensen Egg						
Medium - LOT #J8CZKK	10	-----	36.5°C (±1°C)	3/15/88	5/10/88	No Growth
Virus Pool	10	0.5		|	|	No Growth
Control Pool	10	0.5		3/15/88	5/10/88	No Growth

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool

A. Tertiary African Green Monkey Kidney (AGMK)

	Initial Flasks			0.5 ml per tube Passage #1						
	Lot # 275 (2133)			Lot # 287 (2133)						
	Day 14			Day 14 + 14 = 28						
							Coxsackie A-9 Challenge*			
Material Tested	CPE	Hads	Stain	CPE	Hads	Stain	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Virus Pool	0/2	0/2	0/2	0/40	0/10	0/10	4/4	4/4	4/4	3/4
Control Pool (TCF)	0/2	0/2	0/2	0/40	0/10	0/10	4/4	4/4	4/4	1/4
Control - (1)	0/2	0/2	0/2	0/40	0/10	0/10	4/4	4/4	4/4	3/4
Control - (2)				0/48	0/12	0/12	5/5	5/5	5/5	3/5

* Coxsackie A-9 Challenge Results based on a 3-day incubation at 35°C. All tubes refed with 2 ml of fresh medium prior to challenge.

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool

B. Primary Human Amnion (PHA)

	Initial Flasks Lot # 265 Day: 14			Lot # 279 Day: 14 + 14 = 28			0.5 ml per tube Passage #1 Coxsackie A-9 Challenge*			
Material Tested	CPE	Hads	Stain	CPE **	Hads	Stain	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Virus Pool	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	4/4
Control Pool (TCF)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	3/4
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	2/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	8/8	8/8

* Coxsackie A-9 Challenge Results based on a 5-day incubation at 37°C. All tubes refed with 2 ml of fresh medium prior to challenge.

** On day 24 (14 + 10), all tubes were refed with 2 ml of fresh medium.

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool

C. Human Diploid Fibroblast (MRC-5)

	Initial Flasks			0.5 ml per tube Passage #1						
	Lot # 283 p25			Lot # 294 p23						
	Day 14			Day 14 + 14 = 28						
							Coxsackie A-9 Challenge*			
Material Tested	CPE	Hads	Stain	CPE	Hads	Stain	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Virus Pool	1/2**	0/2	ND	0/40	0/10	0/10	4/4	4/4	3/4	0/4
Control Pool (TCF)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	1/4
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	0/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	7/8	1/8

* Coxsackie A-9 Challenge Results based on a 4-day incubation at 35°C. Tubes refed with 2 ml fresh medium prior to challenge.

** One of 2 virus inoculated flasks exhibited cellular degeneration on day 8 which intensified by day 14. [Note: One additional uninoculated flask exhibited the same cellular degeneration.] This degeneration was not transmissable to the tube subpassages.

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool

D. Primary Rabbit Kidney (PRK)

	Initial Flasks			0.5 ml per tube Passage #1		
	Lot # 264			Lot # 278		
	Day: 14			Day: 14 + 14 = 28		
Material Tested	CPE	Hads	Stain	CPE	Hads	Stain
Virus Pool	0/2	0/2	ND	0/40	0/20	0/20
Control Pool (TCF)	0/2	0/2	ND	0/40	0/20	0/20
Control - (1)	0/2	0/2	ND	0/40	0/20	0/20
Control - (2)				0/24	0/12	0/12

ND = Not done

Table II. Tissue Culture Purity (Safety) Test Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool

E. Whole Human Embryo Fibroblasts (Flow 5000)

	Initial Flasks			0.5 ml per tube Passage #1						
	Lot # 266 p17			Lot # 284 p20						
	Day 14			Day 14 + 14 = 28						
							Coxsackie A-9 Challenge*			
Material Tested	CPE	Hads	Stain	CPE	Hads	Stain	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Virus Pool	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	1/4
Control Pool (TCF)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	2/4
Control - (1)	0/2	0/2	ND	0/40	0/10	0/10	4/4	4/4	4/4	2/4
Control - (2)				0/60	0/12	0/12	8/8	8/8	8/8	3/8

* Coxsackie A-9 Challenge Results based on a 3-day incubation at 35°C. Tubes refed with 2 ml of fresh medium prior to challenge.

ND = Not done

Table III - A. Animal Safety Tests Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool

Animal Species	Inoculum	Vol. (ml)	Route	No.	Lesions, Illness or Deaths over 4 to 6 Week Period	Comments
Adult Rabbits (1500-2500 gms)	Virus Pool	10 x 0.1 1 x 9.0 1 x 0.03	I.D. S.Q. L.Cornea	2	There were no lesions at sites of inoculation. No deaths nor signs of illness or distress.	Test Satisfactory
	Control Pool (TCF)	10 x 0.1 1 x 9.0 1 x 0.03	I.D. S.Q. R. Cornea	2		
	None	—	—	1		
Adult Mice (15-20 grams)	Virus Pool	0.03 0.50	I. Cer. I.P.	20	No deaths nor signs of illness or distress recorded.	Test Satisfactory
	Control Pool (TCF)	0.03 0.50	I. Cer. I.P.	20		
	None	—	—	10		
Suckling Mice (< 24 hours)	Virus Pool	0.01 0.10	I. Cer. I.P.	20	No deaths nor signs of illness or distress over this initial 14-day period.	Test Satisfactory
	Control Pool (TCF)	0.01 0.10	I. Cer. I.P.	20		
	None	—	—	10		
	Dl4 Blind Passage (VP)	0.01 0.10	I. Cer. I.P.	20	No deaths nor signs of illness or distress over this final 14-day period.	100% survival of inoculated sucklings. No evidence of a transmissible agent or of any viral infection.
	Dl4 Blind Passage (CP)	0.01 0.10	I. Cer. I.P.	20		
	Dl4 Blind Passage (None)	0.01 0.10	I.Cer. I.P.	10		
	Dl4 - None	—	—	10		

Table III - B. Animal Safety Tests Results on the Crude Hepatitis A Virus, Strain HM175, Vaccine FI-2, Lots 1A and 1B Pool

Animal Species	Inoculum	Vol. (ml)	Route	No.	Lesions, Illness or Deaths over 4 to 6 Week Period	Comments
Adult Guinea Pigs (350-450 gms)	Virus Pool	0.10 5.00	I. Cer. I.P.	3	No deaths nor signs of illness or disease. Daily rectal temperatures taken over last 3 weeks of observation (+ 0800 hrs) were within normal ranges.	
	Control Pool (TCF)	0.10 5.00	I.Cer. I.P.	3		
	None	—	—	3		

Code	GP#	Mean Temp. (^{o}C)	Temp. Range (^{o}C)
VP-1	736	38.41	38.2 - 38.6
VP-2	737	38.49	38.2 - 38.8
VP-3	738	38.27	38.0 - 38.5
TCF-1	739	38.25	38.0 - 38.4
TCF-2	740	38.29	38.1 - 38.6
TCF-3	741	38.43	38.2 - 38.6
C-1	742	38.45	38.2 - 38.7
C-2	743	38.56	38.2 - 38.8
C-3	744	38.32	38.0 - 38.6

Flow Laboratories

9 May 1988

Dr. Louis Potash
Flow Laboratories, Inc.
McLean, VA 22102

RE: Contract #833/8342

Dear Dr. Potash:

Your four samples, Dengue-4 Virus, Dengue-4 TCF, Hepatitis-A-Virus and Hepatitis A-TCF, which you submitted for the presence of mycoplasma hyorhinis using direct immuno-florescence staining, the DNA Hoechst stain and agar testing were found to be negative.

Sincerely,

Jim Quartey

JQ:kk

Flow Laboratories, Inc.
7655 Old Springhouse Road, McLean, Virginia 22102, USA
Tel: (703) 893-5925 Fax: (703) 356-0774 Telex: 440543 FGI UI
A Flow General Company

MYCOPLASMA TEST RECORD SHEET

Culture Medium	LOT #	No. ml Tested Aerobic	No. ml Tested Anaerobic	Date On Test	Date Off Test	Results
Virus Fluid - LOT # HEPATITIS-A VIRUS = MYC#016						
PPLO Agar	870915	.2	.2	4/5/88	4/20/88	NEGATIVE
PPLO Broth	871015	25.0	~~25.0~~			NEGATIVE
D 5 Subpass to Broth		25.0	~~25.0~~	4/11/88	4/26/88	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	~~25.0~~	4/15/88	5/2/88	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	~~25.0~~	4/20/88	5/5/88	NEGATIVE
to Agar		.2	.2			NEGATIVE
Control Fluid - LOT # HEPATITIS A TCF = MYC#017						
PPLO Agar		.2	.2	4/5/88	4/20/88	NEGATIVE
PPLO Broth		25.0	~~25.0~~			NEGATIVE
D 5 Subpass to Broth		25.0	~~25.0~~	4/11/88	4/26/88	NEGATIVE
to Agar		.2	.2			NEGATIVE
D10 Subpass to Broth		25.0	~~25.0~~	4/15/88	5/2/88	NEGATIVE
to Agar		.2	.2			NEGATIVE
D15 Subpass to Broth		25.0	~~25.0~~	4/20/88	5/5/88	NEGATIVE
to Agar		.2	.2			NEGATIVE

Positive Control (+): M. arginini Negative Control (-): FB29101C070

Date: 5/9/88 Signed: Jim Crowley

REVERSE TRANSCRIPTASE ASSAY

SAMPLE	CPM INCORPORATED rAdT Mg	rAdT Mn	dAdT Mn
1. Dengue-4 TCF (Control fluid)	640	918	
2. Dengue-4 Virus (Challenge Seed, Lot 2)	1,085	1,085	
3. Hepatitis A TCF (control fluid)	318	2,371	
4. Hepatitis A Virus (FI2 Vaccine, Lot 1)	294	1,318	
CONTROLS			
50 µl growth medium	457	1,170	
50 µl culture fluid from cells infected with RSV	297,529	158,305	
10 µl culture fluid from cells infected with MLV + 40 µl growth medium	42,923	2,333,732	

Comments

All samples were negative for polymerase except sample #3. This sample contained a low but significant activity. It is likely that this activity is due to contaminating cell DNA dependent DNA polymerase from cell debris. Since this sample is a control fluid and the activity is not present in the vaccine, we have elected to not perform the dAdT assay to demonstrate this contamination. If you would like, we can perform this assay.

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